(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 October 2003 (30.10.2003)

PCT

(10) International Publication Number
WO 03/088763 A1

(51) International Patent Classification[7]: A23K 1/00

(21) International Application Number: PCT/KR02/00516

(22) International Filing Date: 27 March 2002 (27.03.2002)

(25) Filing Language: Korean

(26) Publication Language: English

(30) Priority Data:
2002/12103 7 March 2002 (07.03.2002) KR

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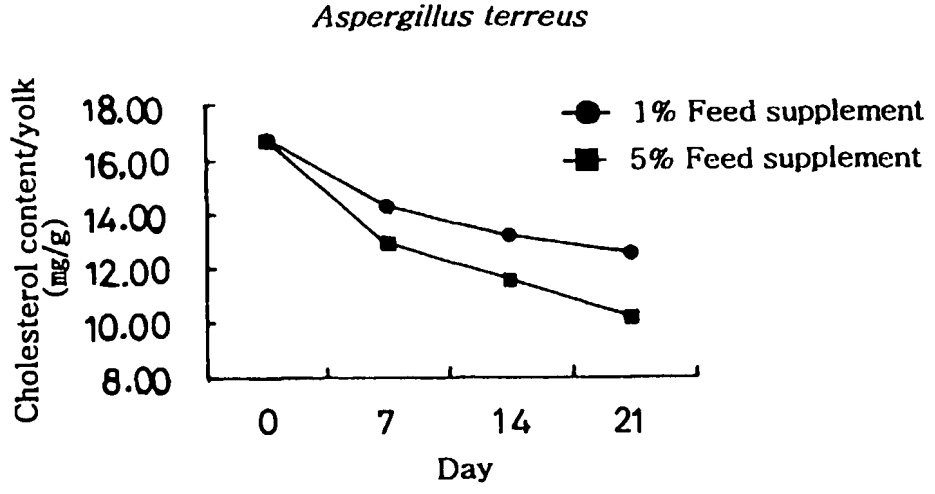
(81) Designated States *(national)*: AE, AT, AU, BG, CA, CH, CN, CO, CR, DE, DK, DM, ES, FI, GB, GH, HU, IL, IN, IS, JP, KG, KZ, LK, MX, NO, NZ, PH, PL, PT, RO, RU, SE, SG, SI, TR, UA, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: METHODS FOR PRODUCING LOW CHOLESTEROL ANIMAL PRODUCTS USING HYPOCHOLESTEROLEMIC FEED SUPPLEMENTS AND PRODUCTS THEREFROM




(57) Abstract: The invention relates to methods for producing low cholesterol animal products, low cholesterol animal products therefrom and hypocholesterolemic feed supplements therefor. Specifically, this invention relates to methods for reducing cholesterol in livestock by using microorganisms comprising said microbial cultures as feed supplements, and low cholesterol animal products, such as meat, poultry, eggs, milk and dairy products, obtained from the produced low cholesterol livestock. Therefore, the invention allows mass production of low cholesterol animal products having 30 % reduced cholesterol content with minimal increase of the production cost, less than 5~10 % of total feed cost enabling the commercialization of low cholesterol animal products using hypocholesterolemic feed supplements.

WO 03/088763 A1

# METHODS FOR PRODUCING LOW CHOLESTEROL ANIMAL PRODUCTS USING HYPOCHOLESTEROLEMIC FEED SUPPLEMENTS AND PRODUCTS THEREFROM

## Technical Field

This invention relates to methods for producing low cholesterol animal products, low cholesterol animal products therefrom and hypocholesterolemic feed supplements therefor. Specifically, this invention relates to methods for reducing cholesterol in livestock by using microorganisms comprising said microbial cultures as feed supplements, and low cholesterol animal products, such as meat, poultry, eggs, milk and dairy products, obtained from the produced low cholesterol livestock.

## Background Art

Cholesterol is a kind of fatty acid in every animal product. It is a constituent of cells and is essential for the synthesis of hormones. An appropriate amount of cholesterol is quite important for health. However, it is important to maintain the suggested level of serum cholesterol since exceed amount of cholesterol can be harmful. Specifically, high serum cholesterol levels is termed hypercholesterolemia (more than 200 mg/dL of blood cholesterol), which is a common chronic disease found in 52% of adults in the world. Hypercholesterolemia is a major risk factor for

arteriosclerosis, which leads *inter alia* to myocardial infarction, angina pectoris, hypertension, and stroke. Currently, coronary artery disease is the leading cause of human mortality in the world although it was the fourth cause of death in 1900s (at most 8%) following pneumonia, influenza, tuberculosis, diarrhea or enteritis.

It is generally accepted that high levels of cholesterol due to a change in the human diet, including more frequent use of animal products, can result in a rise in serum cholesterol and thereby increases the risk of cardiovascular diseases. Therefore there are many efforts to develop the methods for lowering the serum cholesterol level for treatment as well as for prevention of coronary artery disease.

The amount of cholesterol in our body is obtained from diet for adult humans and from biosynthesis. Therefore, serum cholesterol level is affected by the sum of cholesterol from biosynthesis and from dietary intake. Environmental, non-genetic factors, such as dietary habit and diet pattern, significantly affect the individual's intake of cholesterol from food and thereby the total cholesterol level while genetic regulation results the individual's cholesterol biosynthesis relatively constant. Although both genetic and environmental factors can affect the individual's cholesterol level, the excess consumption of animal product, such as meat, poultry, eggs, milk, and dairy products, can cause hypercholesterolemia. However, the consumption of cholesterol-rich animal products increases every year and it seems very difficult to limit the intake of animal products significantly. Therefore, research and development efforts have been directed to

lowering cholesterol level in cholesterol-rich animal products.

One way to reduce the cholesterol content in animal products is to remove the cholesterol by extraction with organic solvents during food processing. The problem with this method is that it requires complicated processing steps and can only be applied to the processed foods although it is effective to reduce the cholesterol content. Therefore, the animal products without processing, such as meat, poultry, eggs, milk, and dairy products, require other approaches, for example, genetic selection or feed supplement.

Genetic selection measures are widely attempted to select the genetically low-cholesterol animals by employing biotechnological tools and genetic selection measures. However, there has been little success in the art in producing low cholesterol animal products by this method since it is both technically difficult and time-consuming.

An alternative to these methods for reducing the cholesterol content of domestic animal products is a dietary measure by feeding high-fiber and low-fat diet or by adding natural hypocholesterolemic components as feed supplements. However, it became clear that it couldn't be used in the commercial application due to their high cost and low efficacy. Thus far, eggs that are representative high-cholesterol foods are only commercially available although the reduction is about 10% and the price is twice compared with ordinary eggs.

Cholesterol is synthesized by multi-step biosynthesis starting from

acetyl-CoA in humans and livestock, warm-blooded animals. The key rate-limiting step in cholesterol biosynthesis is to convert 3-hydroxy-3-methylglutaryl-coenzyme A to mevalonic acid by the key enzyme known as 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase, Formula 1). Therefore, it is known that by inhibition of HMG-CoA reductase inhibit the biosynthesis of cholesterol.

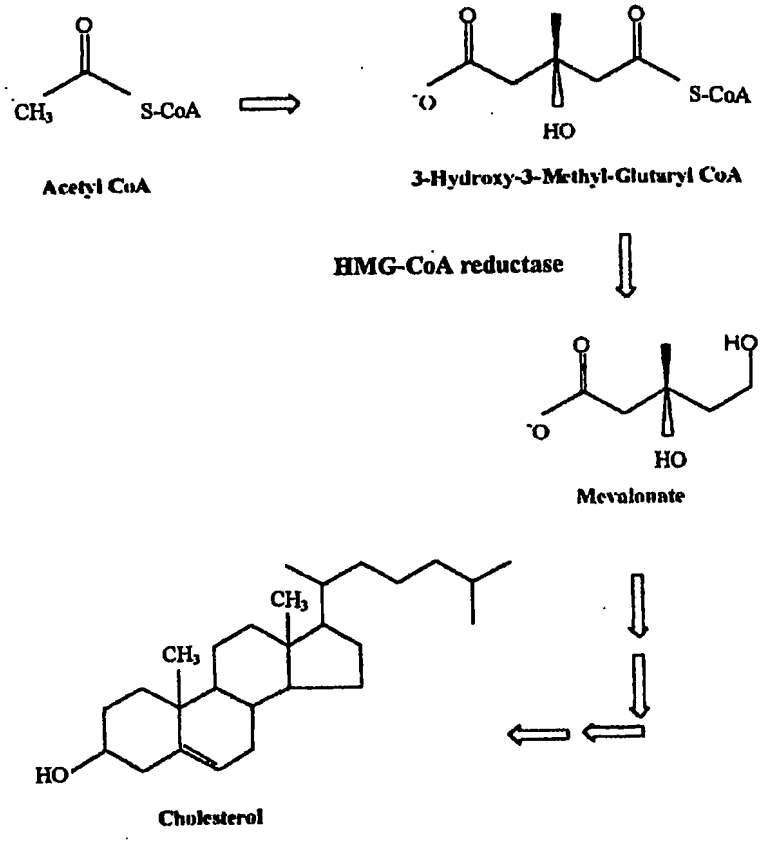
In previous art, it was known that certain mevalonate derivatives, collectively called as statins, inhibit the biosynthesis of cholesterol by inhibition of HMG-CoA reductase and the first such hypocholesterolemic compound discovered was compactin which was isolated from cultures of *Penicillium citrinum* (U.S. Patent No. 3,983,140; 4,049,495; 4,137,322). Thereafter, a hypocholesterolemic compound found to be structurally related to compactin was isolated in fermentation products of several fungal species (known as lovastatin, monacolin K, mevacor, MB530B, MK-803, or MSD803). The isolated active compounds, their derivatives, methods of purification from several genera, and methods of semi-synthetic production from these derivatives have been reported in the art (U.S. Patent Nos. 4,231,938; 4,294,846; 4,294,926; 4,319,039; 4,323,648; 4,342,767; 4,346,227; 4,376,863; 4,420,491; 4,432,996; 4,444,784; 4,450,171; 4,739,073; 5,273,995).

Using microorganisms capable of producing hypocholesterolemic compounds, several statins have been developed as HMG-CoA reductase inhibitors for the treatment of hypercholesterolemia. These include mevastatin (disclosed in U.S. Pat. No. 3,883,140), lovastatin or mevinolin

(disclosed in U.S. Pat. No. 4,231,938), pravastatin (disclosed in U.S. Pat. No. 4,346,227), simvastatin (disclosed in U.S. Pat. Nos. 4,444,784 and 4,450,171), fluvastatin (disclosed in U.S. Pat. No. 4,739,073), atorvastatin (disclosed in U.S. Pat. No. 5,273,995), and derivatives of these compounds, available as prescription drugs.

Formula 1.

Cholesterol biosynthesis



It is hypothesized that the said hypocholesterolemic pharmaceutical compounds can be effective in lowering serum cholesterol levels also in other animals, including livestock, since the regulation of cholesterol biosynthesis is same. However, the said hypocholesterolemic pharmaceutical drugs have a critical problem, that is, extraordinary production cost, to be commercialized as animal feed supplements.

Recently, significant reduction of the cholesterol content of egg yolk was demonstrated by oral administration of purified lovastatin to chickens, as disclosed in U.S. Pat. No.6,177,121. Although the cholesterol-lowering effect was found to be satisfactory when the egg-laying chickens are fed high doses of lovastatin, use of these methods is impractical due to high production cost of low cholesterol eggs, that is, almost greater than 20 times ordinary eggs.

## Disclosure of the Invention

The invention provides low cholesterol animal products, having lower than levels of cholesterol in order to prevent and/or treat hypercholesterolemia and cardiovascular diseases, which are growing serious due to increasing dietary intake of animal products in modern society. In particular, the invention relates methods for producing low cholesterol animal products at production cost, enabling the commercialization thereof.

In other words, this invention provide economic and effective methods to lower serum cholesterol levels in animals by providing hypocholesterolemic feed supplements comprising microbial cultures obtained from cultivating microorganisms capable of producing hypocholesterolemic compounds in fermentation media to make the microorganisms produce the hypocholesterolemic compounds as secondary metabolites. Thus the invention provides economic and effective methods for producing low cholesterol animal products (meat, poultry, eggs, and milk) by supplementing feed with microbial cultures comprising hypocholesterolemic compounds that are effective in reducing cholesterol concentration in animal.

The methods of this invention can be distinguishable from the conventional methods in that effectively low cholesterol animal products can be produced at low cost. In the present invention, microbial culture itself, which produces hypocholesterolemic compounds, is used as hypocholesterolemic feed supplements. In other words, in the present invention, microorganisms are raised in a cheap culture and are directly mixed with ordinary animal feeds, rather than using multi-step processing of isolating hypocholesterolemic compounds from the microbial culture and refining the same. Therefore, additional cost for the production of low cholesterol animal products with hypocholesterolemic feed supplements, including labor cost and material cost, is minimal, less than 5~10% of total feed cost, enabling the commercialization of low cholesterol animal products using hypocholesterolemic feed supplements.

As used herein, the following terms have the following meanings:

"Hypocholesterolemic compounds" is intended to encompass any known or novel microbial compounds, produced naturally or by using genetic engineering, effective in lowering cholesterol content of animals.

"Microbial culture" is intended to encompass single culture or mixed cultures of microorganisms capable of producing hypocholesterolemic compounds, especially including secondary metabolites and microbial cultures.

"Animal," "livestock" and "barnyard animal" is intended to mean any domesticated animals raised for human consumption, including poultry such as chicken, duck, goose and turkey, and mammals such as cows, pigs, sheep, goats and lamb, and fishes.

A "low cholesterol animal" is intended to mean any animal, particularly livestock or barnyard animals, having a blood cholesterol level that is reduced significantly compared to the animal raised in accordance with conventional animal husbandry methods. Examples of low cholesterol animal products include low cholesterol milk, low cholesterol eggs, low cholesterol dairy products and low cholesterol meat.

This invention provides methods for producing low cholesterol animal products wherein animals are fed a feed supplemented with microbial cultures that produce hypocholesterolemic compounds as their secondary metabolites. This invention also provides hypocholesterolemic feed

supplements having said microbial cultures as effective components.

The microbial cultures are any culture of a single type of microorganism that produces hypocholesterolemic compounds, or any mixed cultures thereof. Examples of the microorganism include any microorganisms that belong to genera, but not limited to, *Aspergillus, Penicillium, Paecilomyces, Hypomyces, Doratomyces, Phoma, Eupenicillium, Gymnoascus, Trichoderma, Pleurotus, Monascus, Coniothyrium, Eubacterium,* and *Nocardia.* The microorganisms can be any natural and biotechnological microorganisms that produce hypocholesterolemic compounds without limitation.

The hypocholesterolemic compounds are effective in lowering animal blood cholesterol by inhibiting cholesterol biosynthesis, by inhibiting re-absorption of bile acids along digestive tracts, or by facilitating conversion of cholesterols to bile acids. In particular, the hypocholesterolemic compounds are monacolin K (or mevinolin), monacolin L, monacolin J, monacolin X, monacolin M, lovastatin, compactin, coprostanol and compounds derived therefrom, but not limited to.

Plants and certain microorganisms are known to produce a variety of compounds, known as secondary metabolites through a secondary metabolite pathway. Especially, microorganisms are called as a living chemical factory on earth since it produces almost every chemical. Secondary metabolites have a multiplicity of biological activities, including *for example* antibiotics, anticancer agents, and growth hormones although

certain metabolites are known to be toxic. In contrast, primary metabolite pathway is an essential metabolic pathway for every life forms, including animal, plant, and microorganisms.

Secondary metabolite pathway, however, is expressed in certain plants and microbes at specific environmental condition. Growth in a carbon-deficient is known in the art to promote production of secondary metabolites. Thus, in a preferred embodiment, production of hypocholesterolemic compounds in the microbial cultures of the invention is promoted by incubation in an amino acid-enriched fermentation media after growth in a carbon-enriched propagation media.

Preferably, fermentation media used in the practice of the methods of the invention contain (a) cotton seed extracts as a nitrogen source, (b) any powder or mixture of sugar, rice, corn, potato, and wheat as a carbon source, and (c) sodium (Na), calcium (Ca), iron (Fe), copper (Cu), and manganese (Mn) as trace element components.

More preferably, the said fermentation media contains from about 0.5 to about 1.5% (by weight) cotton seed extract, from about 1.5 to about 4% carbon source, from about 0.1 to about 0.5% NaCl, from about 0.1 to about 0.5% $CaCO_3$, from about 0.01 to about 0.04% $FeCl_3 \cdot 6H_2O$, from about 0.001 to about 0.002% $CuCl_2 \cdot 2H_2O$, from about 0.001 to about 0.002% $MnCl_2 \cdot 4H_2O$, from about 0.002 to about 0.006% $ZnCl_2$, from about 0.001 to about 0.002% $Na_2B_4O_7 \cdot 10H_2O$, and from about 0.001 to about 0.002% $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in water.

An exemplary and non-limiting formulation of the fermentation media provided for use in the methods of the invention is set forth in Table 1. It will be recognized that conditions for secondary metabolite production are not limited to the specific fermentation media disclosed herein.

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Table 1. Composition and characteristics of fermentation media

| Media | Composition (g/liter) |
|---|---|
| PST | 10 g Cotton seed extract (Proflo™), 22. 5 g sucrose, 3 g NaCl, 3g $CaCO_3$, 40 mg $ZnCl_2$, 200 mg $FeCl_3 \cdot 6H_2O$, 10 mg $CuCl_2 \cdot 2H_2O$, 10 mg $MnCl_2 \cdot 4H_2O$, 10 mg $Na_2B_4O_7 \cdot 10H_2O$, 10 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ |
| PRT | 10 g Cotton seed extract (Proflo™), 22. 5 g rice powder, 3 g NaCl, 3g $CaCO_3$, 40 mg $ZnCl_2$, 200 mg $FeCl_3 \cdot 6H_2O$, 10 mg $CuCl_2 \cdot 2H_2O$, 10 mg $MnCl_2 \cdot 4H_2O$, 10 mg $Na_2B_4O_7 \cdot 10H_2O$, 10 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ |
| PCT | 10 g Cotton seed extract (Proflo™), 22. 5 g corn powder, 3 g NaCl, 3g $CaCO_3$, 40 mg $ZnCl_2$, 200 mg $FeCl_3 \cdot 6H_2O$, 10 mg $CuCl_2 \cdot 2H_2O$, 10 mg $MnCl_2 \cdot 4H_2O$, 10 mg $Na_2B_4O_7 \cdot 10H_2O$, 10 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ |
| PPT | 10 g Cotton seed extract (Proflo™), 22. 5 g potato powder, 3 g NaCl, 3g $CaCO_3$, 40 mg $ZnCl_2$, 200 mg $FeCl_3 \cdot 6H_2O$, 10 mg $CuCl_2 \cdot 2H_2O$, 10 mg $MnCl_2 \cdot 4H_2O$, 10 mg $Na_2B_4O_7 \cdot 10H_2O$, 10 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ |
| PWT | 10 g Cotton seed extract (Proflo™), 22. 5 g wheat powder, 3 g NaCl, 3g $CaCO_3$, 40 mg $ZnCl_2$, 200 mg $FeCl_3 \cdot 6H_2O$, 10 mg $CuCl_2 \cdot 2H_2O$, 10 mg $MnCl_2 \cdot 4H_2O$, 10 mg $Na_2B_4O_7 \cdot 10H_2O$, 10 |

| | mg $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ |
|---|---|
| Characteristics | Above fermentation media are carbon-deficient and amino acid-enriched media in order to promote production of secondary metabolites in the hypocholesterolemic microorganisms. |

Specific protocols for preparing hypocholesterolemic feed supplements and low cholesterol animal products by administration of said hypocholesterolemic feed supplement to animals are as follows, which do not limit the scope of this invention.

**Protocol 1. Seed culture**

Microorganisms capable of expressing a secondary metabolite pathway are characterized by the fact that the rate of growth and division decrease significantly when secondary metabolite production is commenced. The purpose of seed culture is to promote growth and division of microorganisms and to repress the secondary metabolite pathway in the propagation media that contains sufficient amounts of carbon sources and grown under aerobic conditions. To this end, microorganisms used for seed culture are first cultivated in propagation media. Compositions for propagation media contain sufficient amounts of carbon sources, which are essential for growth of microorganisms, thereby promoting growth and division of microorganisms. Preferably, for provide sufficient amounts of oxygen, microbial strains are inoculated in 40㎖

propagation media in a 250㎖ baffled flask at a concentration of 0.2% (200㎕ of spore suspension in 100㎖ of propagation media, for instance) and cultured for 2-4 days in appropriate culture condition or using a bioreactor.

**Protocol 2. Cultures to promote production of secondary metabolites**

Once a microbial culture is prepared by the seed culture, the microbial culture is incubated in fermentation media capable of promoting production and secretion of secondary metabolites, as shown in Table 1. The microbial culture is incubated under aerobic conditions to promote the secretion of the secondary metabolites, for example, by growth in a baffled flask for 6 – 10 days. Incubation can be performed using a baffled flask or a bioreactor. In the case of using a baffled flask, 250㎖ fermentation media per 1ℓ flask are provided and agitated at 150 rpm for 6 – 10 days. After production of secondary metabolites is promoted in fermentation media, the cultures containing sufficiently produced secondary metabolites and as many microorganisms as possible, can be used as feed supplements for animal in order to reduce serum cholesterol concentration.

**Protocol 3. Feed supplements composed of microbial culture**

Hypocholesterolemic feed supplement is added to conventional animal feed in an amount of said microbial culture 0.01 – 30% (by weight). Preferably, animal feed can be matured for days, preferably more than 3

days, after supplementation of said microbial culture.

Feeding animals said hypocholesterolemic feed supplement, comprising said microbial culture, can produce low cholesterol animal. A preferred feeding schedule is to feed the animal at least once a day with the supplemented feed for a period of more than two days duration.

In preferred embodiments, supplemented animal feed is fed to the animal at least once a day for at least five days. This feeding will result low cholesterol animals with reduced serum cholesterol levels, 10~30% depending on the extent and duration of feeding.

The invention provides low cholesterol products from low cholesterol animals using the method described in this invention. Said low cholesterol products are all kinds of products obtained from low cholesterol animals such as meat, poultry, eggs, milk and dairy products. Said low cholesterol products also include processed food such as ham, bacon, sausage and other processed meat products, processed dairy products such as butter, cheese or yogurt, and other processed egg products, as well as primary food naturally obtained from low cholesterol animals such as meat, poultry, milk or eggs.

The present invention may be better understood with reference to the accompanying examples that are intended for purposes of illustration only and should not be construed to limit the scope of the invention, as defined by the claims appended hereto.

Brief Description of the Drawings

Figure 1 is a graph demonstrating the hypocholesterolemic effects in chicken eggs after administration of feed supplements comprising *Aspergillus terreus* (ATCC 20542) fermentation products to egg-laying hens in Example 2.

Figure 2 is a graph demonstrating the hypocholesterolemic effects in chicken eggs after administration of feed supplements comprising *Paecilomyces* sp. (M2016) fermentation products to egg-laying hens in Example 3.

Figure 3 is a graph demonstrating the hypocholesterolemic effects in chicken eggs after administration of feed supplements comprising *Penicillium citrinum* (ATCC 20606) fermentation products to egg-laying hens in Example 4.

Figure 4 is a graph demonstrating the hypocholesterolemic effects in chicken eggs after administration of feed supplements comprising *Penicillium brevicompactum* (ATCC 9056) fermentation products to egg-laying hens in Example 5.

Figure 5 is a graph demonstrating the hypocholesterolemic effects in chicken eggs after administration of feed supplements comprising *Hypomyces chrysospermus* (IFO 7798) fermentation products to egg-laying hens in Example 6.

Figure 6 is a graph demonstrating the hypocholesterolemic effects in chicken eggs after administration of feed supplements comprising *Doratomyces nanus* (IFO 9551) fermentation products to egg-laying hens in Example 7.

Figure 7 is a graph demonstrating the hypocholesterolemic effects in chicken eggs after administration of feed supplements comprising *Phoma* sp. (M4452) fermentation products to egg-laying hens in Example 8.

Figure 8 is a graph demonstrating the hypocholesterolemic effects in chicken eggs after administration of feed supplements comprising *Eupenicillium* sp.(M6603) fermentation products to egg-laying hens in Example 9.

Figure 9 is a graph demonstrating the hypocholesterolemic effects in chicken eggs after administration of feed supplements comprising *Gymnoascus umbrinus* (IFO8450) fermentation products to egg-laying hens in Example 10.

Figure 10 is a graph demonstrating the hypocholesterolemic effects in chicken eggs after administration of feed supplements comprising *Trichoderma longibrachiatum* (M6735) fermentation products to egg-laying hens in Example 11.

Figure 11 is a graph demonstrating the hypocholesterolemic effects in chicken eggs after administration of feed supplements comprising *Trichoderma pseudokoningii* (M6828) fermentation products to egg-laying

hens in Example 12.

Figure. 12 is a graph demonstrating the hypocholesterolemic effects in chicken eggs after administration of feed supplements comprising *Pleurotus ostreatus* (ATCC 9415) fermentation products to egg-laying hens in Example 13.

Figure 13 is a graph demonstrating the hypocholesterolemic effects in chicken eggs after administration of feed supplements comprising *Monascus purpureus* (IFO 4513) fermentation products to egg-laying hens in Example 14.

Figure 14 is a graph demonstrating the hypocholesterolemic effects in chicken eggs after administration of feed supplements comprising *Monascus anka* (IFO 6540) fermentation products to egg-laying hens in Example 15.

Best mode for carrying out the Invention

**EXAMPLE 1**

**Preparation of microbial cultures**

1. Seed culture

Fungi were grown on an appropriate solid media by incubation for 5-8 days. Spores were collected using distilled water and stored in 20% glycerol stock solution. Used microorganisms and solid media are shown in Table 2. For producing primary and secondary seed cultures, a

bioreactor was used. First, for primary seed culture, 3.5 x $10^9$ spore was inoculated into 1.5ℓ propagation media (Table 3) in 5ℓ aspirator bottle and cultured for 1-2 days under culture conditions appropriate for growth of the particular fungus. For producing the secondary seed culture, 10% of primary seed culture was inoculated into 60ℓ propagation media in 150ℓ bioreactor and cultured for 1-2 days at 150 rpm at pH 6.5~7.2 under appropriate culture condition.

Table 2. Solid media for seed culture

| Species | Media components (g per 1ℓ ) | Temp. |
|---|---|---|
| *Aspergillus terreus* ATCC 20542 | 20 g malt extract, 5 g peptone, 15 g agar | 26°C |
| *Paecilomyces sp.* M2016 | 300 g diced potato, 20 g glucose, 15 g agar | 24°C |
| *Penicillium citrinum* ATCC 20606 | 300 g diced potato, 20 g glucose, 15 g agar | 24°C |
| *Penicillium brevicompactum* ATCC 9056 | 3 g $NaNO_3$, 1 g $K_2HPO_4$, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 0.01g $FeSO_4 \cdot 7H_2O$, 30 g sucrose, 15 g agar | 24°C |
| *Hypomyces chrysospermus* IFO 7798 | 300 g diced potato, 20 g glucose, 15 g agar | 26°C |
| *Doratomyces nanus* IFO 9551 | 300 g diced potato, 20 g glucose, 15 g agar | 26°C |
| *Phoma sp.* M4452 | 300 g diced potato, 20 g glucose, 15 g agar | 24°C |

| Species | Media components (g per 1ℓ ) | Temp. |
|---|---|---|
| *Eupenicillium sp.* M6603 | 20 g malt extract, 20 g glucose, 1 g peptone, 20 g agar | 20°C |
| *Gymnoascus umbrinus* IFO 8450 | 20 g potato, 20 g carrot, 15 g agar | 26°C |
| *Trichoderma longibrachiatum M6735* | 25 g rabbit food commercial pellet, 15 g agar | 26°C |
| *Trichoderma pseudokoningii* M6828 | 30 g malt extract, 15 g agar | 24°C |
| *Pleurotus ostreatus* ATCC 9415 | 15 g glucose, 5 g peptone, 3 g malt extract, 3 g yeast extract, 20 g agar | 24°C |
| *Monascus purpureus* IFO 4513 | 300 g diced potato, 20 g glucose, 15 g agar | 26°C |
| *Monascus anka* IFO 6540 | 40 g glucose, 10 g peptone, 20 g agar | 26°C |

Table 3. Composition of the propagation media

| Media | Composition (g per 1ℓ ) |
|---|---|
| YEME | 3 g yeast extract, 5 g peptone, 3 g malt extract, 340 g sucrose, 10 g glucose, and 1 g $MgCl_2 \cdot 6H_2O$ |

2. Production of microbial culture containing secondary metabolites.

Once the seed culture using the propagation media is prepared, the microbial culture is inoculated into PST fermentation media having the composition as shown in Table 1. Large scale fermentation cultures were prepared such that 775ℓ fermentation media were placed in a 1,000ℓ bioreactor and 6% of the secondary seed culture was inoculated for

incubating the culture at 80-120 rpm at pH 5.8 – 6.3 for 6-10 days while supplying a sufficient amount of oxygen. The produced microbial culture containing sufficient secondary metabolites and microorganisms was used as animal feed supplement for use in the following examples.

5

**EXAMPLE 2**

**Low cholesterol eggs using *Aspergillus terreus* culture**

1. Feeding hens a feed supplement composed of microbial culture

A culture of *A. terreus* (ATCC Accession No. 20542) was prepared as
10 described in Example 1 above and added to commercial chicken feed and fed to egg-laying hens in every 12 hours for 21 days. Egg-laying hens were 33 weeks old and randomly assigned to one of groups, 6 hens per each cage, for comparative administration of feed supplements ranged 0%, 1%, 5%, 15% and 30% (by weight).

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2. Egg cholesterol analysis

The cholesterol content was analyzed from collected eggs using the following method. Each egg was hard-boiled and the yolk was separated and crumbled. A mixture of chloroform/methanol (15㎖, 2:1 v/v) was
20 added to 1 g of yolk and sonicated for 30 sec. After standing for 30 minutes, the homogeneous solution was filtered through a 0.45 μm

membrane filter. Egg homogenate filtrates were analyzed for cholesterol content using *o*-phthalaldehyde by mixing thoroughly 0.1㎖ of the filtrate, 0.3㎖ of a solution of 33% (w/v) KOH in water, and 3㎖ of 95% ethanol and then saponified for 15 min at 60°C heat block. After saponification, 10㎖ of hexane was added to extract cholesterol and mixed thoroughly. The extracted cholesterol was reacted with enzymatic solution of cholesterol esterase, oxidase or peroxidase and absorbance was determined at 500 nm using a spectrophotometer. Egg cholesterol content was determined by comparison with standard curve. The effect of cholesterol-lowering feed supplements is shown in Table 4 and Figure 1.

Table 4

| Feed supplement | Cholesterol content (mg)/yolk (g) | Cholesterol reduction (%) |
|---|---|---|
| 0% | 16.74 | 0 |
| 1% | 12.56 | 25 |
| 5% | 10.24 | 38.2 |
| 15% | 10.19 | 39.2 |
| 30% | 10.11 | 39.7 |

**EXAMPLE 3**

**Low cholesterol eggs using *Paecilomyces* sp. culture**

A culture of *Paecilomyces* sp. (ATCC Accession No. M2016) was prepared as described in Example 1 and added to commercial chicken feed

1%, 5%, 15%, and 30% respectively. The cholesterol content in eggs produced by these chickens was analyzed as described in Example 2. The effect of cholesterol-lowering feed supplements is shown in Table 5 and Figure 2.

Table 5

| Feed supplement | Cholesterol content (mg)/yolk (g) | Cholesterol reduction (%) |
|---|---|---|
| 0% | 16.74 | 0 |
| 1% | 13.31 | 20.5 |
| 5% | 12.67 | 24.4 |
| 15% | 12.37 | 26.2 |
| 30% | 12.35 | 26.3 |

**EXAMPLE 4**

**Low cholesterol eggs using *Penicillium citrinum* culture**

A culture of *P. citrinum* (ATCC Accession No. 20606) was prepared as described in Example 1 and added to commercial chicken feed 1%, 5%, 15%, and 30% respectively. The cholesterol content in eggs produced by these chickens was analyzed as described in Example 2. The effect of cholesterol-lowering feed supplements is shown in Table 6 and Figure 3.

Table 6

| Feed | Cholesterol content (mg)/yolk | Cholesterol reduction |
|---|---|---|

| supplement | (g) | (%) |
|---|---|---|
| 0% | 16.74 | 0 |
| 1% | 14.42 | 13.9 |
| 5% | 14.15 | 15.5 |
| 15% | 14.06 | 16.1 |
| 30% | 13.96 | 16.7 |

**EXAMPLE 5**

**Low cholesterol eggs using *Penicillium brevicompactum* culture**

A culture of *P. brevicompactum* (ATCC Accession No. 9056) was prepared as described in Example 1 and added to commercial chicken feed 1%, 5%, 15%, and 30% respectively. The cholesterol content in eggs produced by these chickens was analyzed as described in Example 2. The effect of cholesterol-lowering feed supplements is shown in Table 7 and Figure 4.

Table 7

| Feed supplement | Cholesterol content (mg)/yolk (g) | Cholesterol reduction (%) |
|---|---|---|
| 0% | 16.74 | 0 |
| 1% | 14.06 | 16 |
| 5% | 13.47 | 19.6 |
| 15% | 13.32 | 20.5 |
| 30% | 13.30 | 20.6 |

## EXAMPLE 6

### Low cholesterol eggs using *Hypomyces chrysospermus* culture

A culture of *H. chrysospermus* (IFO Accession No. 7798) was prepared as described in Example 1 and added to commercial chicken feed 1%, 5%, 15%, and 30% respectively. The cholesterol content in eggs produced by these chickens was analyzed as described in Example 2. The effect of cholesterol-lowering feed supplements is shown in Table 8 and Figure 5.

Table 8

| Feed supplement | Cholesterol content (mg)/yolk (g) | Cholesterol reduction (%) |
|---|---|---|
| 0% | 16.74 | 0 |
| 1% | 14.26 | 14.8 |
| 5% | 13.58 | 18.9 |
| 15% | 13.49 | 19.5 |
| 30% | 13.39 | 20.1 |

## EXAMPLE 7

### Low cholesterol eggs using *Doratomyces nanus* culture

A culture of *D. nanus* (IFO Accession No. 9951) was prepared as

described in Example 1 and added to commercial chicken feed 1%, 5%, 15%, and 30% respectively. The cholesterol content in eggs produced by these chickens was analyzed as described in Example 2. The effect of cholesterol-lowering feed supplements is shown in Table 9 and Figure 6.

Table 9

| Feed supplement | Cholesterol content (mg)/yolk (g) | Cholesterol reduction (%) |
|---|---|---|
| 0% | 16.74 | 0 |
| 1% | 14.79 | 11.6 |
| 5% | 13.87 | 17.1 |
| 15% | 13.67 | 18.4 |
| 30% | 13.57 | 18.9 |

**EXAMPLE 8**

**Low cholesterol eggs using *Phoma* sp. culture**

A culture of Phoma sp. (ATCC Accession No. M4452) was prepared as described in Example 1 and added to commercial chicken feed 1%, 5%, 15%, and 30% respectively and the cholesterol content in eggs produced by these chickens was analyzed as described in Example 2. The effect of cholesterol-lowering feed supplements is shown in Table 10 and Figure 7.

Table 10

| Feed supplement | Cholesterol content (mg)/yolk (g) | Cholesterol reduction (%) |
|---|---|---|
| 0% | 16.74 | 0 |
| 1% | 14.41 | 13.9 |
| 5% | 13.96 | 16.6 |
| 15% | 13.84 | 17.4 |
| 30% | 13.81 | 17.6 |

**EXAMPLE 9**

**Low cholesterol eggs using *Eupenicillium* sp. culture**

A culture of *Eupenicillium* sp. (ATCC Accession No. M6603) was prepared as described in Example 1 and added to commercial chicken feed 1%, 5%, 15%, and 30% respectively. The cholesterol content in eggs produced by these chickens was analyzed as described in Example 2. The effect of cholesterol-lowering feed supplements is shown in Table 11 and Figure 8.

Table 11

| Feed supplement | Cholesterol content (mg)/yolk (g) | Cholesterol reduction (%) |
|---|---|---|
| 0% | 16.74 | 0 |
| 1% | 14.25 | 14.9 |
| 5% | 13.85 | 17.3 |
| 15% | 13.74 | 18.0 |

| | | |
|---|---|---|
| 30% | 13.63 | 18.6 |

**EXAMPLE 10**

**Low cholesterol eggs using *Gymnoascus umbrinus* culture**

A culture of *G. umbrinus* (IFO Accession No. 8450) was prepared as described in Example 1 and added to commercial chicken feed 1%, 5%, 15%, and 30% respectively. The cholesterol content in eggs produced by these chickens was analyzed as described in Example 2. The effect of cholesterol-lowering feed supplements is shown in Table 12 and Figure 9.

Table 12

| Feed supplement | Cholesterol content (mg)/yolk (g) | Cholesterol reduction (%) |
|---|---|---|
| 0% | 16.74 | 0 |
| 1% | 13.92 | 16.8 |
| 5% | 13.50 | 19.4 |
| 15% | 13.36 | 20.2 |
| 30% | 13.28 | 20.7 |

**EXAMPLE 11**

**Low cholesterol eggs using *Trichoderma longibrachiatum* culture**

A culture of *T. longibrachiatum* (ATCC Accession No. M6735) was prepared as described in Example 1 and added to commercial chicken feed

1%, 5%, 15%, and 30% respectively. The cholesterol content in eggs produced by these chickens was analyzed as described in Example 2. The effect of cholesterol-lowering feed supplements is shown in Table 13 and Figure 10.

Table 13

| Feed supplement | Cholesterol content (mg)/yolk (g) | Cholesterol reduction (%) |
|---|---|---|
| 0% | 16.74 | 0 |
| 1% | 14.85 | 11.3 |
| 5% | 14.68 | 12.3 |
| 15% | 14.66 | 12.5 |
| 30% | 14.61 | 12.8 |

**EXAMPLE 12**

**Low cholesterol eggs using *Trichoderma pseudokoningii* culture**

A culture of *T. pseudokoningii* (ATCC Accession No. M6828) was prepared as described in Example 1 and added to commercial chicken feed 1%, 5%, 15%, and 30%, respectively. The cholesterol content in eggs produced by these chickens was analyzed as described in Example 2. The effect of cholesterol-lowering feed supplements is shown in Table 14 and Figure 11.

Table 14

| Feed supplement | Cholesterol content (mg)/yolk (g) | Cholesterol reduction (%) |
|---|---|---|
| 0% | 16.74 | 0 |
| 1% | 13.67 | 18.4 |
| 5% | 13.43 | 19.8 |
| 15% | 13.47 | 19.6 |
| 30% | 13.22 | 21.1 |

**EXAMPLE 13**

**Low cholesterol eggs using *Pleurotus ostreatus* culture**

A culture of *P. ostreatus* (ATCC Accession No. 9415) was prepared as described in Example 1 and added to commercial chicken feed 1%, 5%, 15%, and 30% respectively. The cholesterol content in eggs produced by these chickens was analyzed as described in Example 2. The effect of cholesterol-lowering feed supplements is shown in Table 15 and Figure 12.

Table 15

| Feed supplement | Cholesterol content (mg)/yolk (g) | Cholesterol reduction (%) |
|---|---|---|
| 0% | 16.74 | 0 |
| 1% | 14.59 | 12.8 |
| 5% | 13.87 | 17.1 |
| 15% | 13.75 | 17.9 |
| 30% | 13.64 | 18.6 |

## EXAMPLE 14

### Low cholesterol eggs using *Monascus purpureus* culture

A culture of *M. purpureus* (IFO Accession No. 4513) was prepared as described in Example 1 and added to commercial chicken feed 1%, 5%, 15%, and 30% respectively. The cholesterol content in eggs produced by these chickens was analyzed as described in Example 2. The effect of cholesterol-lowering feed supplements is shown in Table 16 and Figure 13.

Table 16

| Feed supplement | Cholesterol content (mg)/yolk (g) | Cholesterol reduction (%) |
|---|---|---|
| 0% | 16.74 | 0 |
| 1% | 13.16 | 21.4 |
| 5% | 11.87 | 29.1 |
| 15% | 11.76 | 29.8 |
| 30% | 11.62 | 30.6 |

## EXAMPLE 15

### Low cholesterol eggs using *Monascus anka* culture

A culture of *M. anka* (IFO 6540) was prepared as described in Example 1 and added to commercial chicken feed 1%, 5%, 15%, and 30% respectively. The cholesterol content in eggs produced by these chickens

was analyzed as described in Example 2. The effect of cholesterol-lowering feed supplements is shown in Table 17 and Figure 14.

Table 17

| Feed supplement | Cholesterol content (mg)/yolk (g) | Cholesterol reduction (%) |
|---|---|---|
| 0% | 16.74 | 0 |
| 1% | 13.16 | 21.4 |
| 5% | 11.9 | 28.1 |
| 15% | 11.88 | 29.1 |
| 30% | 11.81 | 29.5 |

These results of examples, from Example 2 to 15, demonstrate that the hypocholesterolemic feed supplements showed about 38% reduction, the most significant cholesterol-lowering effect, after 3-week feeding of microbial culture prepared with *Aspergillus terreus*. *Monascus* sp. also reduced the cholesterol concentration of the eggs about 20 ~ 30%, *Paecilomyces sp., Penicillium Citrinum, Penicillium brevicompactum* about 20% while *Hypomyces, Doratomyces, Phoma, Eupenicillium, Gymnoascus, Trichoderma, Pleurotus* sp. showed about 10 ~ 20% reduction compared to control. Regarding the amount of supplement and its hypocholesterolemic effect, 1% supplementation reduced about 11 ~ 25% and 5% about 12 ~ 38%. Further supplementation to 15% or 30% only marginally reduced the cholesterol amount, indicating the effective amount

of feed supplement is about 1 ~ 5%.

**EXAMPLE 16**

**Cholesterol analysis of chicken sera**

1. Feeding broiler chicken a feed supplement

A microbial culture was prepared as described in Example 1 and the prepared feed supplement was added to commercial chicken feed at 0%, 1% and 5% (by weight) and fed to 6 weeks old chicks, 10 chicks per cage, using a schedule of feeding every 12 hours for 3 weeks. The collected chicken blood was analyzed for cholesterol level.

Analysis of serum cholesterol

Chicken blood was collected and serum was analyzed for cholesterol content. Typically, 0.1㎖ of sera, 0.3㎖ of a 33% (w/v) solution of KOH in water, and 3㎖ of 95% ethanol were mixed thoroughly and saponified for 15 min by heating in a heat block at 60°. After saponification, 10㎖ of hexane was added and mixed thoroughly. The extracted cholesterol was reacted with enzymatic solution of cholesterol esterase, oxidase or peroxidase and absorbance was determined at 500 nm using a

spectrophotometer. Serum cholesterol content was determined by comparison with standard curve. The effect of cholesterol-lowering feed supplements is shown in Table 18.

Table 18

| Strain | Feed supplement (%) | Cholesterol content (mg)/ sera (dl) | Cholesterol reduction (%) |
|---|---|---|---|
| *Aspergillus Terreus* | 0% | 144.8 | 0 |
| | 1% | 117.4 | 19 |
| | 5% | 94.5 | 34.8 |
| *Paecilomyces* sp. | 0% | 144.8 | 0 |
| | 1% | 124.4 | 14.1 |
| | 5% | 104.5 | 27.9 |
| *Penicillium citrinum* | 0% | 144.8 | 0 |
| | 1% | 122.8 | 15.2 |
| | 5% | 103.2 | 28.8 |
| *Penicillium brevicompactum* | 0% | 144.8 | 0 |
| | 1% | 121.9 | 15.8 |
| | 5% | 110.3 | 23.8 |
| *Hypomyces chrysospermus* | 0% | 144.8 | 0 |
| | 1% | 129.6 | 10.5 |
| | 5% | 123.2 | 15 |
| *Doratomyces nanus* | 0% | 144.8 | 0 |
| | 1% | 128.2 | 11.5 |
| | 5% | 119.3 | 17.7 |
| *Phoma* sp. | 0% | 144.8 | 0 |
| | 1% | 131.6 | 9.2 |

| Strain | Feed supplement (%) | Cholesterol content (mg)/ sera (dl) | Cholesterol reduction (%) |
|---|---|---|---|
| | 5% | 123.9 | 14.5 |
| *Eupenicillium* sp. | 0% | 144.8 | 0 |
| | 1% | 125.1 | 13.7 |
| | 5% | 117.8 | 18.7 |
| *Gymnoascus umbrinus* | 0% | 144.8 | 0 |
| | 1% | 135.1 | 6.6 |
| | 5% | 127.1 | 12.3 |
| *Trichoderma longibrachiatum* | 0% | 144.8 | 0 |
| | 1% | 131.4 | 9.3 |
| | 5% | 122.6 | 15.4 |
| *Trichoderma pseudokoningii* | 0% | 144.8 | 0 |
| | 1% | 129.3 | 10.8 |
| | 5% | 121.6 | 16.1 |
| *Pleurotus ostreatus* | 0% | 144.8 | 0 |
| | 1% | 134.2 | 7.4 |
| | 5% | 128.5 | 11.3 |
| *Monascus purpureus* | 0% | 144.8 | 0 |
| | 1% | 121.4 | 16.2 |
| | 5% | 101.5 | 30 |
| *Monascus anka* | 0% | 144.8 | 0 |
| | 1% | 120.8 | 16.6 |
| | 5% | 103.7 | 28.4 |

As shown in Table 18, the hypocholesterolemic feed supplements showed about 34.8% reduction, the most significant cholesterol-lowering

effect, after 3-week feeding of microbial culture prepared with *Aspergillus terreus*. *Monascus* sp. also reduced the cholesterol level about 28 ~ 30%, *Penicillium* sp. about 27 ~ 28% while other supplements from *Paecilomyces*, *Hypomyces*, *Doratomyces*, *Phoma*, *Eupenicillium*, *Gymnoascus*, *Trichoderma*, *Pleurotus* sp. showed 15 ~ 25% reduction compared to the control.

**EXAMPLE 17**

**Cholesterol analysis of pig sera**

310 g of conventional pig feed was supplemented with 0%, 1% and 5% (by weight) of a microbial culture according to Example 1 of the invention and fed to every two young pigs (4 months old) in every morning and evening for 15 days. After feeding, pig sera were analyzed for cholesterol content using the same method as described in Example 16.

Table 19

| strain | Added microbial culture (%) | Cholesterol content (mg)/ sera (dl) | Cholesterol reduction (%) |
|---|---|---|---|
| *Aspergillus terreus* | 0% | 126.5 | 0 |
| | 1% | 100.7 | 20.4 |
| | 5% | 82.1 | 35.1 |
| *Paecilomyces* sp. | 0% | 126.5 | 0 |
| | 1% | 106.3 | 16 |

| strain | Added microbial culture (%) | Cholesterol content (mg)/ sera (dl) | Cholesterol reduction (%) |
|---|---|---|---|
| | 5% | 98.6 | 22.1 |
| *Penicillium citrinum* | 0% | 126.5 | 0 |
| | 1% | 100.7 | 20.4 |
| | 5% | 93.9 | 25.8 |
| *Penicillium brevicompactum* | 0% | 126.5 | 0 |
| | 1% | 106.3 | 16 |
| | 5% | 97.4 | 23.1 |
| *Hypomyces chrysospermus* | 0% | 126.5 | 0 |
| | 1% | 110.6 | 12.6 |
| | 5% | 102.4 | 19.1 |
| *Doratomyces nanus* | 0% | 126.5 | 0 |
| | 1% | 113.7 | 10.2 |
| | 5% | 99.5 | 21.4 |
| *Phoma* sp. | 0% | 126.5 | 0 |
| | 1% | 107.8 | 14.8 |
| | 5% | 97.5 | 23 |
| *Eupenicillium* sp. | 0% | 126.5 | 0 |
| | 1% | 109.6 | 13.4 |
| | 5% | 101.4 | 20 |
| *Gymnoascus umbrinus* | 0% | 126.5 | 0 |
| | 1% | 111.2 | 12.1 |
| | 5% | 102.8 | 18.8 |
| *Trichoderma longibrachiatum* | 0% | 126.5 | 0 |
| | 1% | 109.9 | 13.2 |
| | 5% | 103.7 | 18.1 |

| strain | Added microbial culture (%) | Cholesterol content (mg)/ sera (dl) | Cholesterol reduction (%) |
|---|---|---|---|
| *Trichoderma pseudokoningii* | 0% | 126.5 | 0 |
| | 1% | 106.4 | 15.9 |
| | 5% | 101.5 | 19.8 |
| *Pleurotus ostreatus* | 0% | 126.5 | 0 |
| | 1% | 107.3 | 15.2 |
| | 5% | 98.9 | 21.9 |
| *Monascus purpureus* | 0% | 126.5 | 0 |
| | 1% | 107.5 | 15.1 |
| | 5% | 91.6 | 27.6 |
| *Monascus anka* | 0% | 126.5 | 0 |
| | 1% | 110.5 | 12.7 |
| | 5% | 94.6 | 25.3 |

As shown in Table 19, the hypocholesterolemic feed supplements showed about 35% reduction, the most significant cholesterol-lowering effect, after feeding of culture prepared with *Aspergillus terreus.* Supplements from other microbial cultures showed 15 ~ 28% reduction compared to the control.

**EXAMPLE 18**

**Cholesterol analysis of bovine sera**

Conventional cow feed was supplemented with 0%, 1% and 5% (by

weight) of a microbial culture according to Example 1 of the invention and fed to every two cows (3 years old) in every morning and evening for 20 days. After feeding, cow sera were analyzed for cholesterol content using the same method as described in Example 16. The cholesterol-lowering effect of feed supplements is shown in Table 20.

Table 20

| strain | Added microbial culture (%) | Cholesterol content (mg)/ sera (dl) | Cholesterol reduction (%) |
|---|---|---|---|
| *Aspergillus terreus* | 0% | 192.8 | 0 |
| | 1% | 178.5 | 7.5 |
| | 5% | 169.3 | 12.2 |
| *Paecilomyces* sp. | 0% | 192.8 | 0 |
| | 1% | 182.5 | 5.4 |
| | 5% | 179.8 | 6.8 |
| *Penicillium citrinum* | 0% | 192.8 | 0 |
| | 1% | 181.3 | 6 |
| | 5% | 176.6 | 8.5 |
| *Penicillium brevicompactum* | 0% | 192.8 | 0 |
| | 1% | 180.7 | 6.3 |
| | 5% | 174.3 | 9.6 |
| *Hypomyces chrysospermus* | 0% | 192.8 | 0 |
| | 1% | 184.3 | 4.5 |
| | 5% | 181.4 | 6 |
| *Doratomyces nanus* | 0% | 192.8 | 0 |
| | 1% | 185.2 | 4 |

| strain | Added microbial culture (%) | Cholesterol content (mg)/ sera (dl) | Cholesterol reduction (%) |
|---|---|---|---|
| | 5% | 181.3 | 6 |
| *Phoma* sp. | 0% | 192.8 | 0 |
| | 1% | 184.7 | 4.3 |
| | 5% | 179.7 | 6.8 |
| *Eupenicillium* sp. | 0% | 192.8 | 0 |
| | 1% | 185.3 | 3.9 |
| | 5% | 182.9 | 5.2 |
| *Gymnoascus umbrinus* | 0% | 192.8 | 0 |
| | 1% | 182.5 | 5.4 |
| | 5% | 178.6 | 7.4 |
| *Trichoderma longibrachiatum* | 0% | 192.8 | 0 |
| | 1% | 181.5 | 5.9 |
| | 5% | 175.4 | 9.1 |
| *Trichoderma pseudokoningii* | 0% | 192.8 | 0 |
| | 1% | 180.8 | 6.3 |
| | 5% | 176.9 | 8.3 |
| *Pleurotus ostreatus* | 0% | 192.8 | 0 |
| | 1% | 184.6 | 4.3 |
| | 5% | 179.3 | 7.1 |
| *Monascus purpureus* | 0% | 192.8 | 0 |
| | 1% | 178.4 | 7.5 |
| | 5% | 174.2 | 9.7 |
| *Monascus anka* | 0% | 192.8 | 0 |
| | 1% | 175.0 | 9.3 |
| | 5% | 171.3 | 11.2 |

These results confirmed the cholesterol-lowering effect of hypocholesterolemic feed supplements was about 6 ~11.2 %.

**EXAMPLE 19**

**Analysis of milk cholesterol**

Conventional dairy cow feed was supplemented with 0%, 1% and 5% (by weight) of a microbial culture according to Example 1 of the invention and fed to young milking cows (25 months old), one milking cow which was delivered of 4 weeks ago per each group, twice a day for 3 weeks. After feeding, milk samples were analyzed for cholesterol content using the following method. Typically, 0.1㎖ of milk, 0.3㎖ of a 33% (w/v) solution of KOH in water, and 3㎖ of 95% ethanol were mixed thoroughly and saponified for 15 min by heating in a heat block at 60°. After saponification, 10㎖ of hexane was added and mixed thoroughly. The extracted cholesterol was reacted with enzymatic solution of cholesterol esterase, oxidase or peroxidase and absorbance was determined at 500 nm using a spectrophotometer. Cholesterol content was determined by comparison with standard curve.

Table 21

| Strains | Added microbial culture (%) | Cholesterol content (mg)/ milk (dl) | Cholesterol reduction (%) |
|---|---|---|---|

| Strains | Added microbial culture (%) | Cholesterol content (mg)/ milk (dl) | Cholesterol reduction (%) |
|---|---|---|---|
| *Aspergillus terreus* | 0% | 10.16 | 0 |
| | 1% | 7.26 | 28.5 |
| | 5% | 6.52 | 35.9 |
| *Paecilomyces* sp. | 0% | 10.16 | 0 |
| | 1% | 8.47 | 16.7 |
| | 5% | 7.82 | 23.1 |
| *Penicillium citrinum* | 0% | 10.16 | 0 |
| | 1% | 8.39 | 17.5 |
| | 5% | 8.02 | 21.1 |
| *Penicillium brevicompactum* | 0% | 10.16 | 0 |
| | 1% | 8.48 | 16.5 |
| | 5% | 7.94 | 21.9 |
| *Hypomyces chrysospermus* | 0% | 10.16 | 0 |
| | 1% | 8.82 | 13.2 |
| | 5% | 8.53 | 16.1 |
| *Doratomyces nanus* | 0% | 10.16 | 0 |
| | 1% | 8.61 | 15.3 |
| | 5% | 8.29 | 18.5 |
| *Phoma* sp. | 0% | 10.16 | 0 |
| | 1% | 8.71 | 14.3 |
| | 5% | 8.56 | 15.8 |
| *Eupenicillium* sp. | 0% | 10.16 | 0 |
| | 1% | 9.02 | 11.3 |
| | 5% | 8.88 | 12.6 |
| *Gymnoascus* | 0% | 10.16 | 0 |

| Strains | Added microbial culture (%) | Cholesterol content (mg)/ milk (dl) | Cholesterol reduction (%) |
|---|---|---|---|
| *umbrinus* | 1% | 8.59 | 15.5 |
| | 5% | 8.21 | 19.2 |
| *Trichoderma longibrachiatum* | 0% | 10.16 | 0 |
| | 1% | 8.59 | 15.5 |
| | 5% | 8.12 | 20.1 |
| *Trichoderma pseudokoningii* | 0% | 10.16 | 0 |
| | 1% | 8.65 | 14.9 |
| | 5% | 8.48 | 16.6 |
| *Pleurotus ostreatus* | 0% | 10.16 | 0 |
| | 1% | 8.32 | 18.2 |
| | 5% | 8.08 | 20.5 |
| *Monascus purpureus* | 0% | 10.16 | 0 |
| | 1% | 8.09 | 20.4 |
| | 5% | 7.87 | 22.6 |
| *Monascus anka* | 0% | 10.16 | 0 |
| | 1% | 8.12 | 20.1 |
| | 5% | 7.69 | 24.4 |

As shown in Table 21, the hypocholesterolemic feed supplements showed about 35.9% reduction, the most significant cholesterol-lowering effect, after feeding of culture prepared with *Aspergillus terreus*. Supplements from other microbial cultures showed 16 ~ 24% reduction compared to the control.

Industrial Applicability

As described herein, the invention provides low cholesterol animal products (meat, poultry, eggs, milk) from low cholesterol animal, using hypocholesterolemic feed supplements comprising microbial cultures in fermentation media and containing hypocholesterolemic compounds effective for reducing cholesterol concentration in an animal. This allows commercialization of low cholesterol animal products, having 30% reduced cholesterol concentration, with minimal increase of production cost, about 5 ~ 10%. Therefore, this invention has utilities in producing low cholesterol animal products marketable to the individuals with health concerns, such as hypercholesterolemia and coronary artery disease, as well as to the individuals caring for prevention of hypercholesterolemia.

What is claimed is:

1. A method of producing low cholesterol animal products by adding a microbial culture producing hypocholesterolemic compounds to animal feeds and feeding the same to livestock.

2. The method of claim 1, wherein the hypocholesterolemic compounds are cholesterol-lowering compounds using secondary metabolites contained in a microbial culture lowering animal blood cholesterol by inhibiting cholesterol biosynthesis, by inhibiting re-absorption of bile acids along digestive tracts, or by facilitating conversion of cholesterols to bile acids.

3. The method of claim 1, wherein the hypocholesterolemic compound is monacolin K (mevinolin), monacolin L, monacolin J, monacolin X, monacolin M, lovastatin, compactin, coprostanol or mixtures and cholesterol-lowering derivatives thereof.

4. The method of claim 1, wherein the microbial culture is a single or mixed culture comprising microorganisms of genera *Aspergillus, Penicillium, Paecilomyces, Hypomyces, Doratomyces, Phoma, Eupenicillium, Gymnoascus, Trichoderma, Pleurotus, Monascus, Coniothyrium, Eubacterium* or *Nocardia*.

5. The method of any one of claims 1 through 4, wherein the low cholesterol animal products have reduced cholesterol by 10% compared to ordinary animal products.

6. The method of any one of claims 1 through 4, wherein animal feed is supplemented with from 0.01 – 30% by weight of the microbial culture and the animal is fed the feed at least once per day for more than 2 days.

7. Low cholesterol animal products produced according to the method in claim 1.

8. Low cholesterol animal products of claim 7, wherein the animal products are meat, eggs, whole milk or dairy products.

9. Processed food prepared using the low cholesterol animal products in claim 7 and claim 8.

10. A hypocholesterolemic feed supplement for reducing blood cholesterol of animal, comprising a microbial culture producing hypocholesterolemic compounds as an effective component.

11. The hypocholesterolemic feed supplement of claim 10, wherein the hypocholesterolemic compounds are cholesterol-lowering compounds using secondary metabolites contained in a microbial culture lowering animal blood cholesterol by inhibiting cholesterol biosynthesis, by inhibiting re-absorption of bile acids along digestive tracts, or by facilitating conversion of cholesterols to bile acids.

12. The hypocholesterolemic feed supplement of claim 10, wherein the hypocholesterolemic compound is monacolin K (mevinolin), monacolin L, monacolin J, monacolin X, monacolin M, lovastatin, compactin, coprostanol or mixtures and cholesterol-lowering derivatives thereof.

13. The hypocholesterolemic feed supplement of claim 10, wherein the microbial culture is a single or mixed culture comprising microorganisms of genera *Aspergillus, Penicillium, Paecilomyces, Hypomyces, Doratomyces, Phoma, Eupenicillium, Gymnoascus, Trichoderma, Pleurotus, Monascus, Coniothyrium, Eubacterium* or *Nocardia*.

14. The hypocholesterolemic feed supplement of any one of claims 10 through 13, wherein the microbial culture is prepared from fermentation media containing (a) cotton seed extracts as a nitrogen source, (b) any powder or mixture of sugar, rice, corn, potato, and wheat as a carbon source, and (c) sodium (Na), calcium (Ca), iron (Fe), copper (Cu), and manganese (Mn) as trace element components.

15. The hypocholesterolemic feed supplement of claim 14, wherein the fermentation media comprises 0.5 ~ 1.5% cotton seed extracts, 1.5 ~ 4% of a carbon source, 0.1 ~ 0.5% NaCl, 0.1 ~ 0.5% $CaCO_3$, 0.01 ~ 0.04% $FeCl_3 \cdot 6H_2O$, 0.001 ~ 0.002% $CuCl_2 \cdot 2H_2O$, 0.001 ~ 0.002% $MnCl_2 \cdot 4H_2O$, 0.002 ~ 0.006% $ZnCl_2$, 0.001 ~ 0.002% $Na_2B_4O_7 \cdot 10H_2O$, 0.001 ~ 0.002% $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in water.

16. An animal feed composition comprising the hypocholesterolemic feed supplement of claim 10.

17. The animal feed composition of claim 16, wherein the animal feed is supplemented with from 0.1 – 30% of the microbial culture.

DRAWINGS

FIG 1

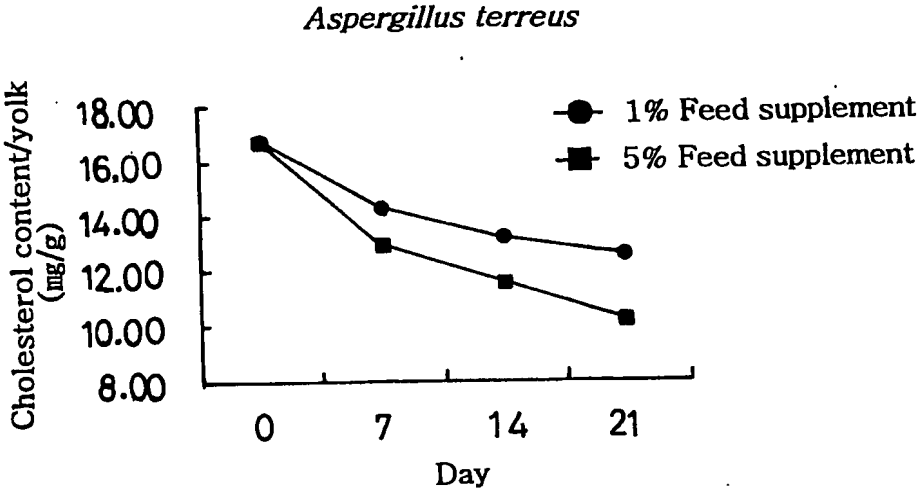



FIG 2

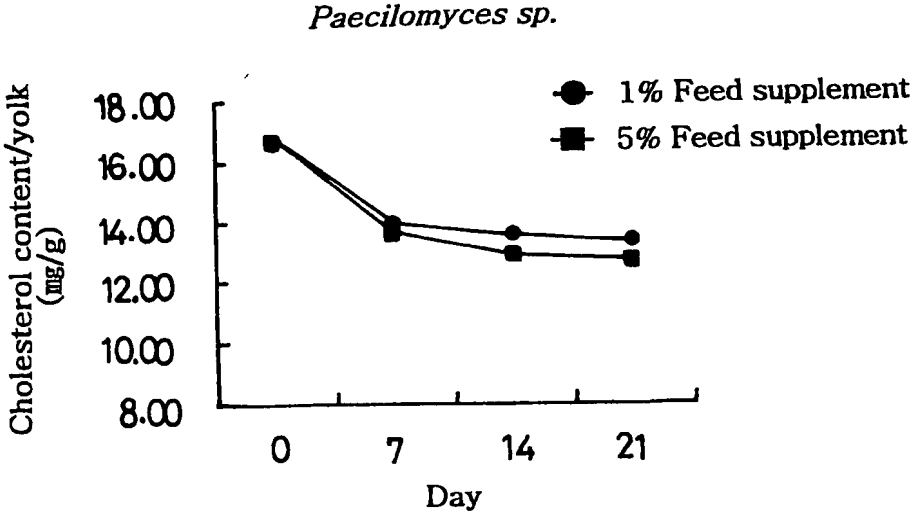


FIG 3

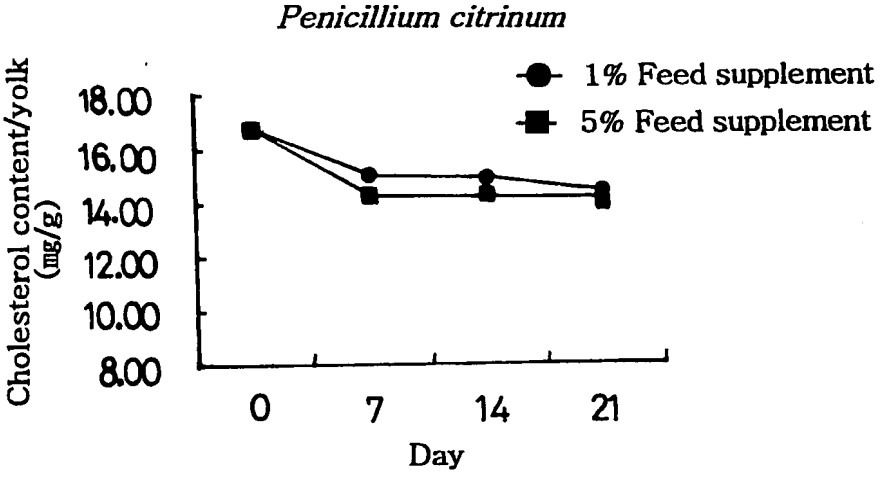

Penicillium citrinum
1% Feed supplement
5% Feed supplement
Cholesterol content/yolk (mg/g)
18.00
16.00
14.00
12.00
10.00
8.00
0
7
14
21
Day

FIG 4

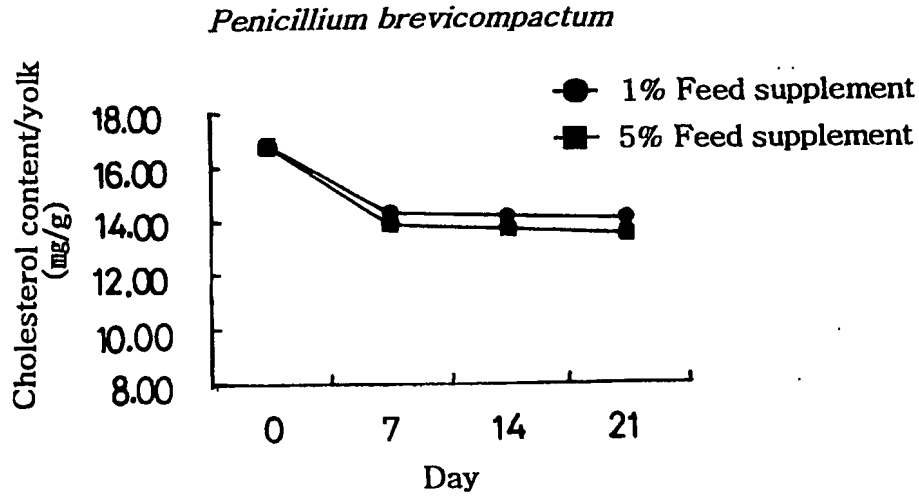

Penicillium brevicompactum
1% Feed supplement
5% Feed supplement
Cholesterol content/yolk (mg/g)
18.00
16.00
14.00
12.00
10.00
8.00
0
7
14
21
Day

FIG 5

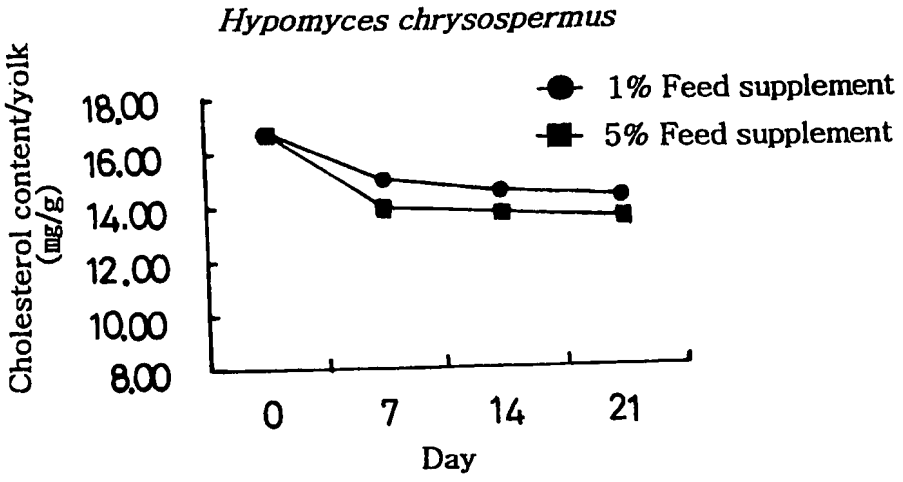

Hypomyces chrysospermus
Cholesterol content/yolk (mg/g)
18.00
16.00
14.00
12.00
10.00
8.00
0
7
14
21
Day
1% Feed supplement
5% Feed supplement

FIG 6

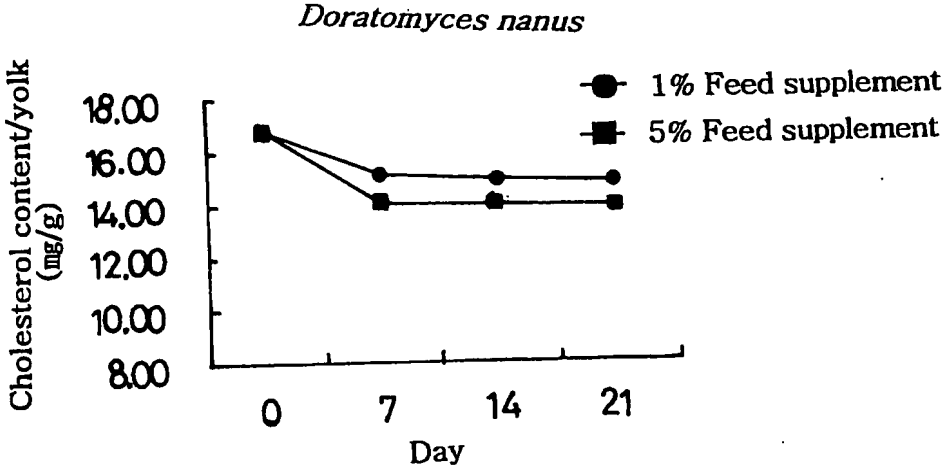

Doratomyces nanus
Cholesterol content/yolk (mg/g)
18.00
16.00
14.00
12.00
10.00
8.00
0
7
14
21
Day
1% Feed supplement
5% Feed supplement

FIG 7

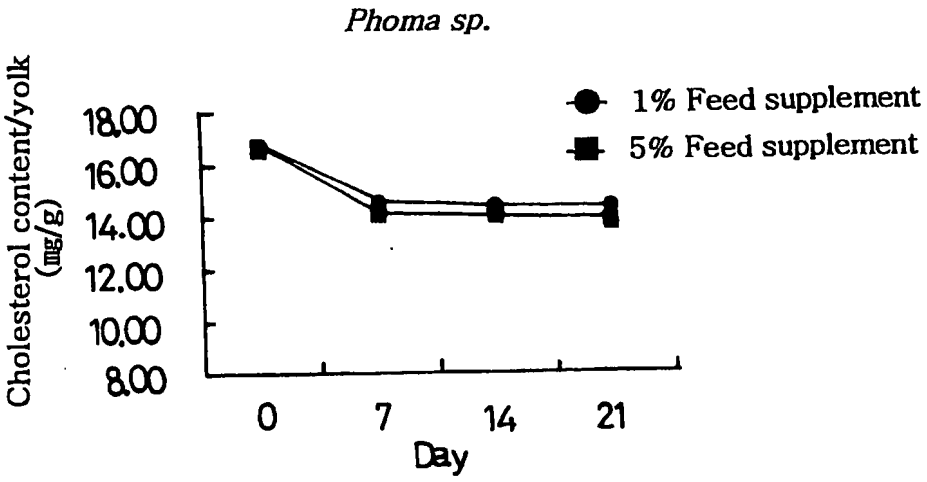

Phoma sp.
Cholesterol content/yolk (mg/g)
18.00
16.00
14.00
12.00
10.00
8.00
0
7
14
21
Day
1% Feed supplement
5% Feed supplement

FIG 8

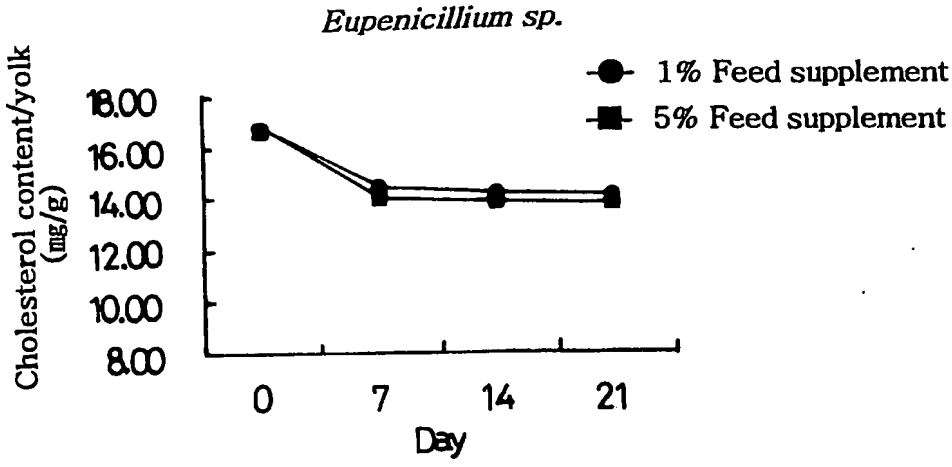

Eupenicillium sp.
Cholesterol content/yolk (mg/g)
18.00
16.00
14.00
12.00
10.00
8.00
0
7
14
21
Day
1% Feed supplement
5% Feed supplement

FIG 9

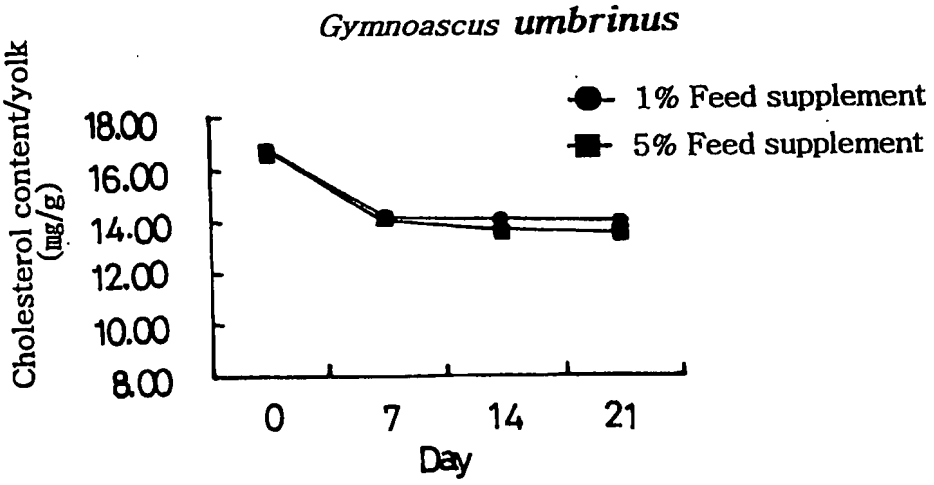

Gymnoascus umbrinus
Cholesterol content/yolk (mg/g)
18.00
16.00
14.00
12.00
10.00
8.00
0
7
14
21
Day
1% Feed supplement
5% Feed supplement

FIG 10

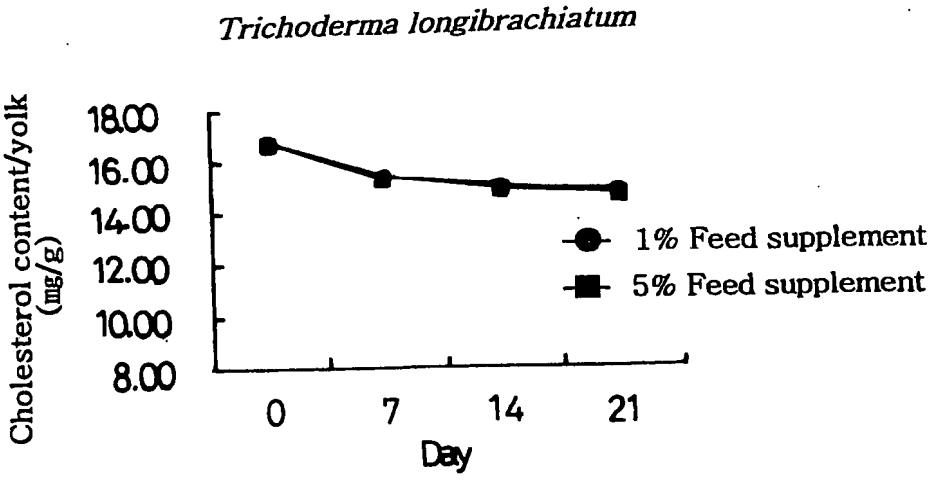

Trichoderma longibrachiatum
Cholesterol content/yolk (mg/g)
18.00
16.00
14.00
12.00
10.00
8.00
0
7
14
21
Day
1% Feed supplement
5% Feed supplement

FIG 11

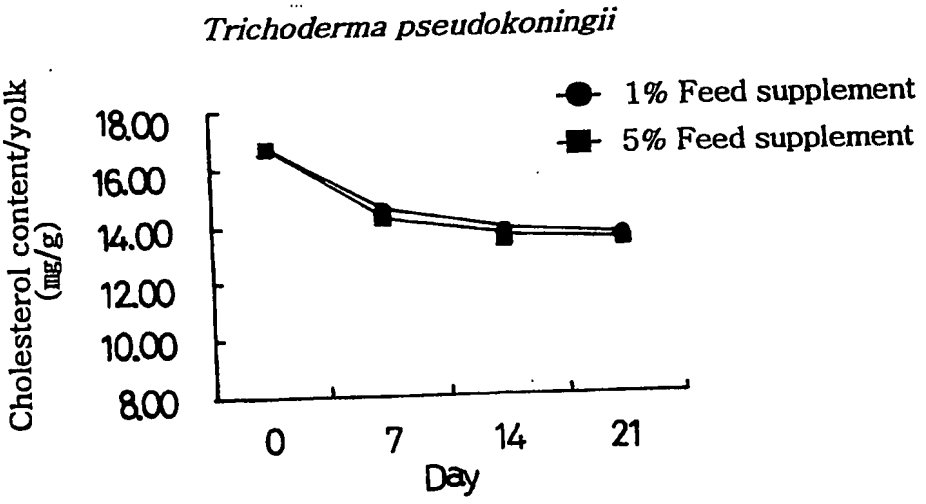

Trichoderma pseudokoningii
Cholesterol content/yolk (mg/g)
18.00
16.00
14.00
12.00
10.00
8.00
0
7
14
21
Day
1% Feed supplement
5% Feed supplement

FIG 12

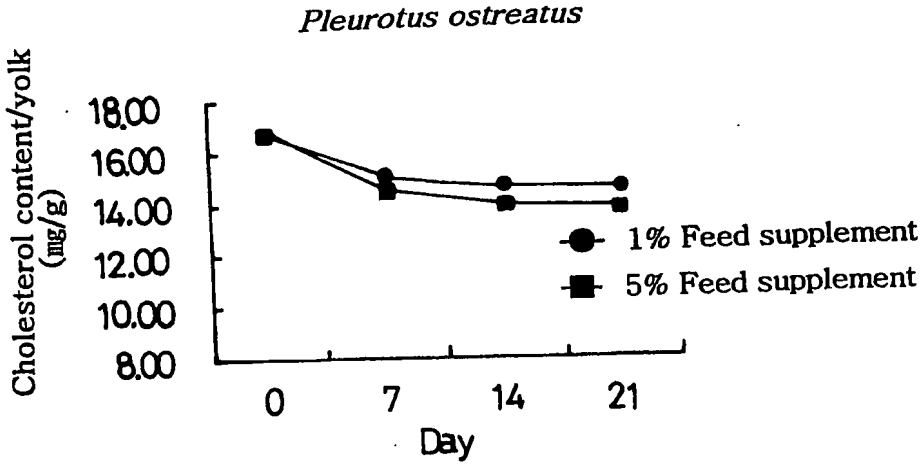

Pleurotus ostreatus
Cholesterol content/yolk (mg/g)
18.00
16.00
14.00
12.00
10.00
8.00
0
7
14
21
Day
1% Feed supplement
5% Feed supplement

FIG 13

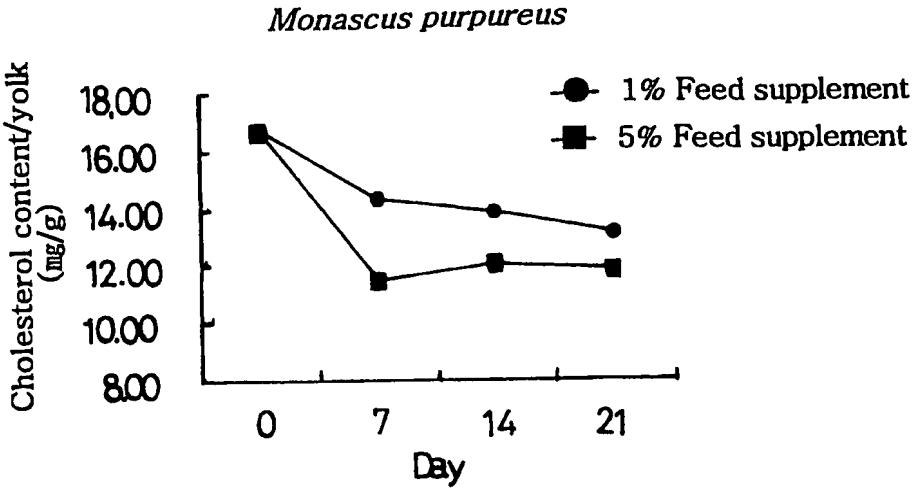
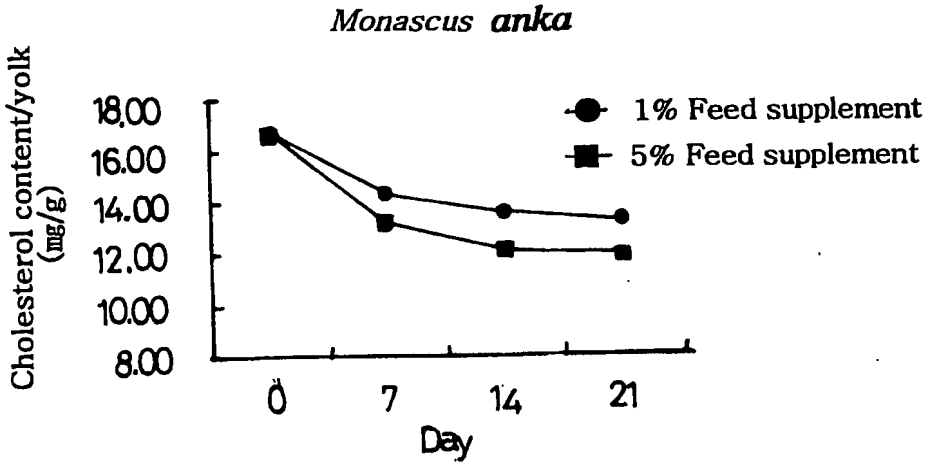

Monascus purpureus
Cholesterol content/yolk (mg/g)
18.00
16.00
14.00
12.00
10.00
8.00
0
7
14
21
Day
1% Feed supplement
5% Feed supplement

FIG 14


Monascus anka
Cholesterol content/yolk (mg/g)
18.00
16.00
14.00
12.00
10.00
8.00
0
7
14
21
Day
1% Feed supplement
5% Feed supplement

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR02/00516

**A. CLASSIFICATION OF SUBJECT MATTER**

**IPC7 A23K 1/00**

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
IPC7 A23K1/00,1/16,1/165; C12N1/14, C07C69/732

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean patents and applications for inventions simce 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
KIPASS, PAJ

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>—<br>Y | KR 2001-69775 A (KIM, SOON-DONG) 25 JULY 2001<br>see the whole document | 1-13, 16, 17<br>---<br>14, 15 |
| Y | US 5691191 A (Sherry Darlene Heins, Davis) 25 NOVEMBER 1997<br>see coulmn 2, line 25-37 | 14, 15 |
| A | US 4921710 A (Donald C. Beitz) 1 MAY 1990<br>see the abstract;<br>see coulmn 1, line 25-56 | 1-17 |
| A | JP 60130548 A (ENDO AKIRA) 12 JULY 1985 | 3, 12 |
| A | KR 1999-82623 A (BIO-FEED CO. LTD) 25 NOVEMBER 1999<br>see page 1,7; claim 22 | 1-17 |

☐ Further documents are listed in the continuation of Box C.  ☒ See patent family annex.

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 28 NOVEMBER 2002 (28.11.2002) | 29 NOVEMBER 2002 (29.11.2002) |


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HONG, Soon Pyo
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## INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR02/00516

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| KR 2001-69775 A | 25/07/2001 | NONE | |
| US 5691191 A | 25/11/1997 | NONE | |
| US 4921710 A | 01/05/1990 | NONE | |
| JP 60130548 A | 12/07/1985 | NONE | |
| KR 1999-82623 A | 25/11/1999 | AU 721811 B2 | 13/07/2000 |
| | | EP 881886 A1 | 09/12/1998 |
| | | CN 1225556 A | 11/08/1999 |
| | | CA 2245964 AA | 21/08/1997 |
| | | WO 9729645 A1 | 21/08/1997 |
| | | AU 1819097 A1 | 02/09/1997 |